METHOD FOR ADENOVIRUS PURIFICATION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of PCT/EP2019/060431 filed on Apr. 24, 2019, which claims priority to Great Britain Application No. 1806758.7 filed on Apr. 25, 2018, the entire contents of which are hereby incorporated by reference herein.

FIELD OF THE INVENTION

[0002] The present invention relates to a method for virus purification. The present invention provides downstream processes for purification of adenovirus from cell culture harvest. More closely, it relates to a method for adenovirus purification using a virus capture and a virus polishing step.

BACKGROUND OF THE INVENTION

[0003] Human adenoviruses have been classified into six species (A to F) with 55 known serotypes causing a wide range of illnesses, from mild respiratory infections in young children (known as the common cold) to life-threatening multi-organ disease in people with a weakened immune system. Adenoviruses are common pathogens that are widely used experimentally and in completed and ongoing clinical trials for gene delivery in oncology, cardioangiology, and regenerative medicine and as vaccine vectors.

[0004] Adenovirus is evaluated as vaccine delivery system in many preclinical and clinical studies of various infectious diseases (Kallel, H. and Kamen, A. A. Large-scale adenovirus and poxvirus-vectored vaccine manufacturing to enable clinical trials. *Biotechnol J* 10, 741-747 (2015). Adenovirus is also explored as a potential viral vector for gene therapy and as an oncolytic virus. To enhance productivity and to produce more effective and safer vaccines, several generations of recombinant adenovirus vectors have been developed (Appaiahgari, M. B. and Vrati, S. Adenoviruses as gene/vaccine delivery vectors: promises and pitfalls. *Expert Opin Biol Ther* 15, 337-351 (2015). The most studied adenovirus vector is the first generation of recombinant adenovirus serotype 5 (AdV5), making this a suitable system for development of a process for adenovirus production.

[0005] Chromatographic techniques are used for adenovirus purification but the results have often been insufficient in respect of purity, yield and capacity.

[0006] Thus, there is still a need to find better and more efficient ways for adenovirus purification, especially in those case the virus is to be used as adenoviral gene delivery vectors.

SUMMARY OF THE INVENTION

[0007] The present invention provides an efficient method for adenovirus purification with high capacity and high yield.

[0008] The invention relates to A method for adenovirus purification comprising the following steps: a) capturing adenovirus from an adenovirus-containing cell culture harvest on an anion exchanger resin; b) eluting said adenovirus with a shallow conductivity gradient with an increasing salt concentration of 15-25%, preferably 18-20%; c) adding said eluted adenovirus to a shell bead resin comprising a porous shell and a porous core, wherein the core is provided with hydrophobic interaction ligands and the shell is not provided with any ligands; and d) eluting said adenovirus from said shell bead resin in the flow through, wherein the adenovirus eluted in step d) comprises less than 1 ng/ml host cell protein (HCP).

[0009] Preferably the salt is NaCl and the gradient is increasing 18-20% and the salt concentration is 0-700 mM, such as 480-570 mM NaCl as in the Examples below or any interval between 0-700 mM that corresponds to a shallow gradient increasing 18-20% from the starting salt concentration.

[0010] Preferably the anion exchange resin is multimodal Capto Q ImpRes anion exchange resin and the shell bead resin is Capto Core 700 resin.

[0011] The anion exchange resin is packed in a column and the shell bead resin is packed in another column, and the eluted adenovirus is added (loaded) to the shell bead resin in a volume corresponding to 15-30 column volumes (CV), preferably 25-30 CV, of the column comprising shell bead resin. The porosity of the core and shell may be the same or different.

[0012] The adenovirus eluted in step d) is very pure and comprises no detectable impurities. The level of host cell protein (HCP) is undetectable or less than 1 ng/ml.

[0013] As shown in the experimental section, the recovery of the adenovirus eluted in step d) is 80-100%.

[0014] A preferred use of the adenovirus is as an adenoviral vector for cell therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIG. **1** shows an overview of two two-step chromatography processes for adenovirus purification, Process of the invention a Reference Process.

[0016] FIG. **2** shows chromatograms from scale-up the Process of the invention, using (A) Capto Q ImpRes for the capture step and (B) Capto Core 700 for the polishing step.

[0017] FIG. **3** shows chromatograms for the Reference process, using A) Sepharose QXL for the capture step and (B) Sepharose 4 Fast Flow for the polishing step.

DETAILED DESCRIPTION OF THE INVENTION

[0018] This invention describes the development of downstream chromatography steps for purification of adenovirus from HEK293 cell culture harvest. After screening of 10 different anion exchange or multimodal resins and 1 anion exchange membrane in small scale, two resins were selected based on highest dynamic binding capacity. Process conditions were optimized and polishing steps were included for further reduction of impurities to meet regulatory requirements. The performance was compared in small scale for a first anion exchange capture step followed by a polishing by either size exclusion or by using Capto core multimodal resin. A reference process and the developed downstream Process of the invention were compared in larger scale, using 3 L cell culture harvest. Although the overall process outcome shows a comparable performance between the developed two-step chromatography processes, the load capacity was approximately 150-fold higher for the polishing step of the inventive process by the use of Capto core 700 resin instead of traditional size exclusion.